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Report

Rapid Displacement of a Monoecious Plant Lineage Is Due to Pollen Swamping by a Dioecious Relative

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Summary

Interspecific hybridization is recognized as a potentially destructive process that represents a major threat to biodiversity [1–3]. The rate of population displacement by hybridization can be rapid, but underlying mechanisms are often obscure. One hypothesis is that a species may be driven to extinction by interspecific gene flow, or pollen swamping, when hybrids are inviable or sterile [1–3]. Here, we document the rapid movement of two zones of contact between monoecious hexaploid and dioecious diploid populations of the wind-pollinated plant *Mercurialis annua* (Euphorbiaceae) in northeastern and northwestern Spain, where diploids have displaced hexaploids by about 80 and 200 km, respectively, over a period of four decades. By using experimental mating arrays, we show that hybridization is highly asymmetrical in favor of the diploids, mainly because they disperse substantially more pollen, as expected in a comparison between an obligate outcrosser and a facultative selfer [4–6]. Self-fertilization, which is expected to reduce the proportion of sterile hybrids produced in mixed ploidy populations [7–11], allowed the hexaploids to avoid the effects of pollen swamping only slightly, and in a density-dependent manner. Our results thus provide a mechanistic explanation for the rapid movement of both contact zones of *M. annua* in Spain.

Results and Discussion

Movement of Diploid-Hexaploid Contact Zones

A sharp transition occurs between diploid and hexaploid populations of the ruderal plant *Mercurialis annua* in Spain, with the hexaploid populations distributed south and west of the diploids in northeastern and northwestern Spain, respectively [12]. We surveyed these regions in 2003 using mitotic root tip squashes and flow cytometry across both contact zones (see Supplemental Data available with this article online) and recorded contact zones between diploid and hexaploid lineages between L'Hospitalet de l'Infant and L'Ametlla de Mar in northeastern Spain and between Baio and Muros in northwestern Spain (Figure 1). These locations are approximately 80 and 200 km southwest and west, respectively, of their recorded positions in 1959 [12], with diploids having displaced hexaploids in both zones.

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Durand [12] reported the location of the Spanish and other ploidy transitions in *M. annua* after analysis of meiotic cell squashes, and he referred precisely to their positions in 1959 relative to specific towns and villages. His survey in northeastern Spain was sufficiently detailed to identify hexaploid populations in three locations within the diploid range; these isolated populations have apparently avoided contact with the diploid advance and persisted through 2003, albeit diminished in size. We attempted to verify the historical location of the contact zones through inspection of herbarium specimens of *M. annua* collected in Spain over the past 100 years (details available from authors), but we found none that could confirm or contradict Durand's [12] locality data. Durand [12] recorded the position of northwestern contact zone somewhat less precisely than in the northeast. However, here we estimated the displacement of hexaploid populations conservatively at 200 km.

Pollen Swamping as a Cause of Contact-Zone Movements

Hybrid progeny between parents of different ploidy are commonly of low fitness [13, 14]. We thus hypothesized that the *M. annua* diploid-hexaploid contacts in Spain are tension zones, maintained by positive frequency-dependent selection [15], with their observed movement caused by asymmetrical pollen swamping. To test this hypothesis, we established artificial mating arrays and estimated the proportion of tetraploid hybrids in the seedling progeny of diploid and hexaploid mothers by means of flow cytometry of progeny leaf preparations (see Supplemental Data). Each array consisted of a lattice of 64 plants, comprising hexaploid and diploid *M. annua* individuals from the northeastern contact zone, and planted at a ratio of 1:3, 1:1, or 3:1, and at high (15 cm between plants) or low (50 cm between plants) densities, in a fully crossed design, with a 1:1 sex ratio for the dioecious diploid component. To account for the direction of displacement of hexaploids by diploids, we expected to find a higher hybridization rate in progeny produced by hexaploids than by diploids.

There was a striking difference between ploidal levels in the proportion of hybrids in their progeny ($p < 0.001$, Figure 2). Very few progeny produced by diploids were hybrids. In contrast, a substantial proportion of hexaploid progeny were tetraploid hybrids, particularly at high densities and at high diploid frequencies ($p < 0.01$, Figure 2). The diploid males produced much more pollen than the monoecious hexaploids ($p < 0.001$; Table 1, and see below), as expected for a comparison between an obligate outcrosser and a facultative selfer [4–6]. This likely allowed them to swamp the stigmas of both hexaploids and diploid female plants with their pollen. The hybrid progeny were substantially less fertile than nonhybrid progeny in both their male ($p < 0.0005$) and female ($p < 0.001$; Table 2) functions, so the reproductive potential of the hexaploids was severely compromised in all arrays. Taken together, the asymmetric hybridization

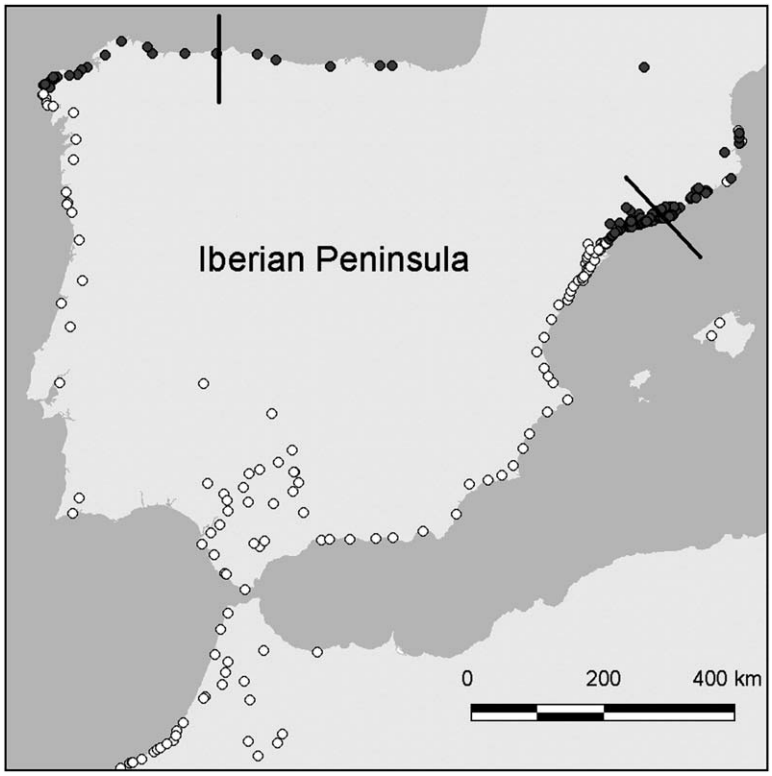

Figure 1. Surveyed Populations of *M. annua* in Iberia and Morocco

Unfilled points represent hexaploid populations, and filled points, diploid. The approximate positions of the contact zones in 1959 are shown by solid lines.

found in our experiments and the low fertility of tetraploid hybrids provide a plausible explanation for the rapid displacement of hexaploids by diploids at two distinct tension zones of *M. annua* in northern Spain.

To our knowledge, this is the first account of a moving diploid-polyploid tension zone for any plant or animal and the first study to have linked it directly to the outcome of positive frequency-dependent selection. In general, interspecific hybridization can be a destructive process and is recognized as a major current threat to biodiversity [1–3, 16]. For example, vigorous hybrids outcompete their progenitors in *Spartina* [17] and *Rorippa* [18], and asymmetric introgression between related species has been recorded in several other species (e.g., [19–22]). However, moving hybrid zones in which one native species displaces another are more unusual. Of the few cases that have been identified (e.g., [23–26]), the rates of movement of the *M. annua* contact zones would appear to be at an upper extreme.

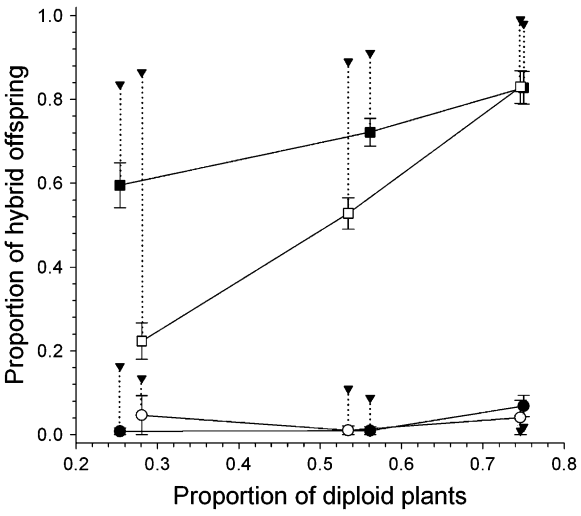

Figure 2. Experimental Results

The proportion of hybrid offspring produced by diploid plants (circles) and hexaploid plants (squares) in populations with differing proportions of diploid plants, spaced at 44.4 plants m^{-2} (filled symbols) and 4 plants m^{-2} (open symbols). Bars show one standard error. Triangles denote the expected hybridization rate for the respective array, assuming random mating (observed and expected values are linked by dotted lines); see text for details.

Self-Fertilization as a Mitigating Effect on Pollen Swamping

Although hybridization rates of hexaploids were high, they were lower than predicted by measures of relative pollen production alone by between 16% and 74% (goodness of fit, $\chi^2_{[5]} = 90.0$, $p < 0.0001$; Figure 2). It has frequently been noted that self-fertilization should reduce rates of hybridization between lineages of different ploidal level [8–11]. As monoecious hexaploid individuals of *M. annua* are self-compatible and produce a full complement of seed by selfing in the absence of mates [27], we predicted that pollen swamping in our experiments might have been mitigated by self-fertilization in hexaploids, assortative mating, or both processes acting together.

In order to disentangle the possible effects of selfing and assortative mating in our arrays, we constructed a model, assuming that mating is the result of lottery-model competition among pollen grains of different type around each stigma, as we might expect under wind pollination. Let A_i be the area occupied by a focal plant and

Table 1. Attributes of Mating Arrays on Harvest

	Low-Density Arrays			High-Density Arrays		
Area (m^2)	16	16	16	1.44	1.44	1.44
N_f	9	15	25	8	16	24
N_m	7	16	23	8	16	24
N_h	40	27	15	47	25	16
L_d	0.131 (0.041)	0.148 (0.013)	0.121 (0.008)	0.169 (0.020)	0.109 (0.014)	0.162 (0.010)
L_h	0.004 (0.0005)	0.014 (0.002)	0.002 (0.0004)	0.010 (0.001)	0.009 (0.001)	0.009 (0.001)
2x mass (g)	7.36 (1.04)	21.06 (2.38)	10.28 (0.93)	5.76 (0.66)	12.04 (1.38)	10.63 (0.94)
6x mass (g)	3.82 (0.40)	10.96 (1.95)	7.14 (1.19)	2.69 (0.18)	6.89 (0.60)	3.44 (0.43)

Showing: area of array (A); frequency of female diploids (N_f), male diploids (N_m), and monoecious hexaploids (N_h); male allocation of diploid males (L_d) and hexaploid plants (L_h); and mean mass of diploids (both sexes) and hexaploids. Standard errors are shown in parentheses for all mean values. In some arrays, numbers deviated from planting ratios due to mortalities.

two tiers of its nearest neighbors; let $\pi_{h,i}$ and $\pi_{d,i}$ be the total amount of pollen produced by the neighboring hexaploids and diploids in the array, respectively; and let $p_{h,i}$ be the pollen production of the focal plant, if hexaploid. We define γ_h and γ_d to be the relative advantage experienced by pollen from diploids over that from hexaploids in fertilizing ovules produced by hexaploids and diploids, respectively, after pollination (i.e., $\gamma_h < 1$ and $\gamma_d > 1$ reflect assortative mating). Finally, let α be the relative advantage of self pollen over nonself pollen in pollinating a hexaploid stigma; $\alpha > A_i^{-1}$ will then reflect an advantage for self pollen, e.g., due to proximity between self anthers and stigmas. With these assumptions, the probability of hybridization of the ith ovule on a focal hexaploid or diploid individual, respectively, is given by

$$H_{h,i} = \frac{\pi_{d,i}\gamma_h A_i^{-1}}{p_{h,i}\alpha + \pi_{h,i}A_i^{-1} + \pi_{d,i}\gamma_h A_i^{-1}}. \quad (1a)$$

$$H_{d,i} = \frac{\pi_{h,i}}{\pi_{h,i} + \pi_{d,i}\gamma_d}. \quad (1b)$$

We measured $H_{h,i}$, $H_{d,i}$, $\pi_{h,i}$, $\pi_{d,i}$, $p_{h,i}$, and A_i for a sample of ovules in each array (see Supplemental Data and Table 1) and estimated $\alpha = 1.639 \pm 0.580$, $\gamma_h = 0.288 \pm 0.045$, and $\gamma_d = 28.49 \pm 24.97$ (least squares estimate ± SE) by a nonlinear least-squares regression of equations 1a and 1b, as appropriate, with Gauss-Newton iterations (see Supplemental Data). As $\gamma_h < 1$ and $\gamma_d > 1$, pollen from diploid plants was evidently less successful at fertilizing ovules on hexaploids but more successful at fertilizing ovules on diploids than their estimated pollen production would have predicted. This could be due to a degree of prezygotic reproductive isolation. The high value of γ_d (though note the high standard error) is also likely to be due to the fact that diploid males disperse pollen from an erect inflorescence stalk (peduncle), whereas hexaploid male flowers are more or less concealed in the leaf axils. Our estimate for α indicates that self-pollen grains were more successful at fertilizing ovules than their relative numbers in a well-mixed pollen cloud would predict in the low-density arrays ($\alpha > A_i^{-1} = 0.19$), but not in the high-density arrays ($\alpha < A_i^{-1} = 2.12$), likely due to proximity between self anthers and stigmas.

We estimated the probability of selfing of hexaploid ovule, i, as

$$S_{h,i} = \frac{p_{h,i}\alpha}{p_{h,i}\alpha + \pi_{h,i}A_i^{-1} + \pi_{d,i}\gamma_h A_i^{-1}}. \quad (2)$$

At high and low densities of 44.4 and 4.0 plants m^{-2}, respectively, the estimated mean selfing probabilities were 0.03 and 0.25. As expected for a wind-pollinated plant [28–30], mating was therefore strongly density dependent, with reduced selfing and greater susceptibility to hybridization at high plant densities.

Of course, self-fertilization may cause inbreeding depression [11, 31–33], and this might compromise the advantage gained by avoiding hybridization. However, if δ is the level of inbreeding depression in selfed progeny, f_h is the frequency of hexaploids in the population, $\lambda = p_d/p_h$, and hybrids are completely sterile, it is easily shown that increased selfing will be beneficial in a mixed population when

$$\delta < 1 - \frac{f_h\gamma}{2\lambda(1 - f_h) + 2f_h\gamma}. \quad (3)$$

It is clear from this that selfing will be increasingly likely to benefit hexaploid fitness as the frequency of diploids (i.e., $1 - f_h$) increases. In hexaploid *M. annua*,

Table 2. Fertility of *Mercurialis annua* Progeny from Diploid and Hexaploid Plants in the Mating Arrays, Showing Reduced Fertility of Hybrids

		Proportion Seed Set		Pollen Viability	
Ploidy of Ovule Donor	Ploidy of Pollen Donor	Mean	n	Mean	n
2	2	0.433 (0.014)	40	0.918 (0.017)	40
2	6	0.233 (0.080)	6	—[1]	—[1]
6	2	0.002 (0.001)	48	0.044 (0.026)	48
6	6	0.454 (0.031)	39	0.786 (0.046)	39

Female function measured by proportion of seed set in flowers, and male function measured by pollen viability. Germination rates for seed produced by each ploidal level within arrays varied between 21% and 62% but did not follow a discernable pattern with any of the variables. Standard errors are given in parentheses.
[1] No male flowers produced.

$\delta < 0.5$ (S.M. Eppley and J.R.P., unpublished data), so that selfing should be an effective strategy to protect ovules from hybridization.

An ability to self-fertilize should relax constraints on the establishment of new polyploid lineages while they occur at low frequencies in sympatry with their diploid progenitors, because the diploids otherwise threaten to swamp their stigmas with pollen ("minority cytotype exclusion" [7]) [8–11]. Our results here illustrate the potential importance of this ability. Although the dioecy-monoecy contrast in sexual system between ploidy races of *M. annua* is unusual in plants, a similar difference has also been observed between hermaphroditic polyploid *Empetrum hermaphroditum* and dioecious diploid *E. nigrum* [34], and artificially induced autopolyploids in dioecious *Silene* and *Rumex* also gave rise to hermaphrodite progeny [35]. More commonly, an ability to self-fertilize may evolve through the breakdown of (gametophytic) self-incompatibility systems [36, 37] or through changes in phenology or flower morphology brought about by chromosome doubling (reviewed in [38, 39]). In other cases, increased selfing may be easier to evolve from outcrossing under conditions of reduced inbreeding depression after polyploidization [11, 40]. Interactions between the mating system and the contaminating effect of pollen swamping might therefore be quite frequent.

Our results suggest that in the case of *M. annua*, the extent to which selfing mitigates the effect of pollen swamping will depend on how often natural plant densities are low. *M. annua* is a colonizing species, and populations may frequently pass through low-density bottlenecks with correspondingly high selfing rates in hexaploids, making them relatively immune to pollen swamping from diploids. However, established populations of *M. annua* can be very dense, reaching values of up to approximately 2000 plants m^{-2} [41]. In such populations, selfing rates in hexaploids will be negligible.

The Future of Hexaploid *M. annua*

Given the recent movement of the diploid-hexaploid contact zones of *M. annua* and the mechanism we have invoked to explain it, the future demise of hexaploid *M. annua* would seem to be inevitable, all else being equal. However, four factors could impede the future advance of the diploids. First, further movement might be prevented by local adaptation in the hexaploid cytotype. However, under the growing conditions of our arrays, we found that diploid females produced significantly more and larger seeds than hexaploid monoecious individuals, and diploids were larger (Table S1). This general superiority of diploids over hexaploids was also confirmed in reciprocal transplant experiments in situ across the northeastern contact zone (R.J.A.B. and J.R.P., unpublished data). We therefore expect ecophysiological divergence between diploids and hexaploids to increase rather than decrease the rate of diploid advance.

Second, it has been hypothesized that monoecious individuals should enjoy the advantage of reproductive assurance over males and females during colonization, because they produce a full complement of seed by self-fertilization in the absence of mates [41, 42]; this advantage might prevent the expansion of dioecious diploids in areas where population turnover is frequent. Recently, we found strong indirect support for differences in population turnover between dioecious and monoecious populations of *M. annua* (S.M. Eppley and J.R.P., unpublished data), but the implications for contact-zone dynamics need to be investigated theoretically.

Third, in southern Spain, many hexaploid populations of *M. annua* are androdioecious, where males cooccur with hermaphrodites [12]. We might expect hexaploid males in this region to dilute the amount of diploid pollen in mixed ploidy populations and impede the advance of the diploids. However, this scenario seems unlikely, because male frequencies in androdioecious populations are low, and total pollen production in these populations ranges between about 0.2 and 0.8 of that of dioecious populations [41]. Array experiments that included males at different frequencies and densities would throw valuable light onto this possibility. Finally, movement of the contact zones may simply be slowed or stopped in a region of low population density, as expected for tension zones generally [43]. The implications of asymmetrical density-dependent mating when populations are ephemeral and when they occur in landscapes that may differ in their habitat availability raise interesting questions that require further theoretical and empirical work to answer.

Supplemental Data

Supplemental Data include one table and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/10/996/DC1/.

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